

IN THE SPECIFICATION

Please substitute the following numbered paragraphs for the paragraphs by the same number originally filed:

[0036] Figure 3 Figures 3A-D shows show the structure of immunoglobulin enhancer genes. Fig. 3A shows the nucleotide (SEQ ID NO:1) Nucleotide and protein (SEQ ID NO:21) sequence of the mouse alpha-1-antitrypsin. Fig. 3B shows the nucleotide (SEQ ID NO:2) and protein (SEQ ID NO:28) of the mouse and endothelial monocyte-activating polypeptide I gene products. Fig. 3C shows an alignment of AAT nucleotide sequences from hamster (SEQ ID NO:7), human (SEQ ID NO:8), mouse (SEQ ID NO:1), rabbit (SEQ ID NO:9), rat (SEQ ID NO:10), sheep (SEQ ID NO:11), and a consensus sequence (SEQ ID NO:12); the mouse AAT polypeptide sequence (SEQ ID NO:21), and an alignment of AAT polypeptide sequences from: mouse (SEQ ID NO:21), rat (SEQ ID NO:22), human (SEQ ID NO:23), sheep (SEQ ID NO:24), hamster (SEQ ID NO:25), rabbit (SEQ ID NO:26) along with a consensus sequence (SEQ ID NO:27). Fig. 3D shows an alignment of EMAPI nucleotide sequences from rabbit (SEQ ID NO:13), dog (SEQ ID NO:14), human (SEQ ID NO:15), rat (SEQ ID NO:16), pig (SEQ ID NO:17), mouse (SEQ ID NO:2) and a consensus sequence (SEQ ID NO:18); the mouse EMAPI polypeptide sequence (SEQ ID NO:28), and an alignment of EMAPI polypeptide sequences from: dog (SEQ ID NO:29) mouse (SEQ ID NO:28), rabbit (SEQ ID NO:30), human (SEQ ID NO:31), rat (SEQ ID NO:32), pig (SEQ ID NO:33) along with a consensus sequence (SEQ ID NO:34).

[0072] To confirm that these proteins or lack thereof are involved in regulating antibody production, we have isolated the full-length cDNAs for each gene to be cloned into the sense and/or antisense direction of a mammalian expression vector. Figure 3 shows the isolated cDNA and predicted encoded polypeptide for the murine alpha-1-anti-trypsin (Fig 3A) and the murine endothelial monocyte-activating polypeptide I (Fig 3B). Because of their possible role in regulating antibody or immunoglobulin production in mammalian systems we performed a blast search and identified AAT homologs from

hamster (SEQ ID NO:7) , human (SEQ ID NO:8), rabbit (SEQ ID NO:9), rat (SEQ ID NO:10), and sheep (SEQ ID NO:11) (Fig 3C) and EMAPI homologs from rabbit (SEQ ID NO:~~12~~ 13), dog (SEQ ID NO:~~13~~ 14), human (SEQ ID NO:~~14~~ 15), rat (SEQ ID NO: ~~15~~ 16), and pig (SEQ ID NO:~~16~~ 17) (Fig 3D) that can be of use for enhancing antibody/immunoglobulin production from cells derived from any of these respective species. The consensus sequence for AAT homologs (SEQ ID NO:12) is shown in Fig. 3C, and the consensus sequence for EMAPI homologs (SEQ ID NO:18) is shown in Fig. 3D.

[0077] The associated lack of AAT and EMAPI expression with enhanced antibody production from producer strains is useful for screening for high antibody production strains. To demonstrate this utility, we generated monoclonal antiserum against the murine AAT and murine EMAPI protein using polypeptides for AAT (SEQ ID NO:17-AAT: ((C)QSPIFVGKVVDPYHK) (SEQ ID NO:19)) and for EMAPI SEQ ID NO:18-EMAPI ((C)IACHDSFIQTSQKRI) (SEQ ID NO:20) derived from their respective translated proteins using methods used by those skilled in the art. We next tested the ability of these antisera to detect protein in the conditioned medium of H6 and H34 cells since both proteins are secreted polypeptides. Briefly, conditioned medium from 10,000 cells were prepared for western blot analysis to assay for steady state protein levels (Figure 4). Briefly, cells were pelleted by centrifugation and 100uls of conditioned supernatant were resuspended in 300 ul of SDS lysis buffer (60 mM Tris, pH 6.8, 2% SDS, 10% glycerol, 0.1 M 2-mercaptoethanol, 0.001% bromophenol blue) and boiled for 5 minutes. Proteins were separated by electrophoresis on 4–12% NuPAGE gels (for analysis of Ig heavy chain. Gels were electroblotted onto Immobilon-P (Millipore) in 48 mM Tris base, 40 mM glycine, 0.0375% SDS, 20% methanol and blocked at room

temperature for 1 hour in Tris-buffered saline (TBS) plus 0.05% Tween-20 and 5% condensed milk. Filters were probed with a 1:1000 dilution of mouse anti-AAT or mouse anti-EMAP antiserum in TBS buffer for 1 hour at room temperature. Blots were washed three times in TBS buffer alone and probed with a 1:10000 dilution of sheep anti-mouse horseradish peroxidase conjugated monoclonal antibody in TBS buffer and detected by chemiluminescence using Supersignal substrate (Pierce). Experiments were repeated in duplicates to ensure reproducibility. Figure 4 shows a representative analysis where low producer H6 parental cells (Lane 1) had robust, steady-state AAT protein levels while no expression was observed in H34 over producer cells (Lane 2). These data suggest a method for screening of cell lines for expression of AAT or EMAP to identify high-titer producer strains that can be used to manufacture high levels of antibody or recombinant polypeptides.

Please enter the attached Sequence Listing following paragraph [0078] and before the claims.